PATENT SPECIFICATION

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COMPLETE SPECIFICATION

Improvements in or relating to Injectable Preparations

We, GLAXO LABORATORIES LIMITED, a British Company of Greenford, Middlesex, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention concerns a novel type of injectable composition of use in medicine and incorporating one or more high-molecular weight physiologically active substances.

In investigating the adjuvant properties of oily substances in injectable vehicles, it has previously only been proposed to use anhydrous oil suspensions or emulsions of the simple or multiple water-in-oil or oil-in-water type. Adjuvants of this type, however are often unstable on storage or too thick easily to be injected. Furthermore, an emulsion or a suspension of a hydrophilic substance in oil, being opaque, is a somewhat inelegant product and is difficult to measure acvcurately in a syringe.

The present invention is particularly concerned with the problem of formulating high molecular weight, hydrophilic, oil-insoluble physiologically active substances such as bacterial toxoids and other antigens in an oily medium without losing the desired physiological acticity and without encountering the above-described difficulties associated with the use of emulsions.

We have now found that optically clear active material and water in a physiologically solutions of non-ionic amphiphilic surface acceptable lipophilic dispersion medium (in contrast to the previously proposed cloudy or opaque emulsions) have the remarkable property of holding in solution a wide variety of high molecular weight hydrophilic substances such as antigens, polysaccharides, nucleic acids etc. and of releasing such physiologically active substances on injection. Such media are also particularly advantageous as delayed release vehicles for insoluble antigens such as living or dead micro-organisms.

According to the present invention we provide injectable compositions consisting of or containing water maintained in solution (as hereinafter defined) in a physiologically acceptable lipophilic dispersion medium, which is liquid at body temperature, by means of one or more non-ionic, amphiphilic, physiologically acceptable surface active substances, said water having associated therewith one or more physiologically-active micro-organisms and/or physiologically active molecular weight hydrophilic oil — insoluble materials.

The term "solution" is used herein to indicate a system in which the water is held by the surface-active material in a continuous lipophilic dispersion medium and that the resulting systems are virtually optically clear when viewed by transmitted light, as distinct from emulsions or suspensions which are cloudy or opaque, unless additional components such as thickening agents are added which themselves lend opacity to the solutions.

Optically clear solutions of the kind utilised in the present invention include all colloidal solutions wherein the aggregates of water and surface-active materials are of a diameter below about 800 Å and thus no longer cause appreciable opacity to visible light. The size range of the aggregates is thus approximately 50—800 Å; above about 100 Å within this range the solutions are sometimes termed "microemulsions". This is in contradistinction to normal or macroemulsions wherein the droplet size is of the order of 2,000 Å. The conditions required for formation of solutions (including the so-called microemulsions) are such that the resulting aggregates are thermodynamically stable, in contrast to macroemulsions which are necessarily thermodynamically unstable, even though the equilibrium conditions of phase separation may be greatly delayed (L. I. Osipow, J. Soc. Cosmetic Chem. 1963 *14*, 277—288; L.M. Prince, J. Colloid an Interface Science, 23, 165—173).

[*Price 4s. 6d.*]

The physiologically active material may itself be solubilised in the above sense to give a clear solution or may remain in suspension; micro organisms of course will always be in suspension. Even suspension of the above kind may sometimes be virtually optically clear in transmitted light if the refractive index of the organisms is close to that of the medium.

The new compositions, being in general optically clear solutions, are not only more elegant in appearance than emulsions or suspensions but have manufacturing advantages. They are far easier to sterilise because filtration methods may be employed and the preparations are, in general, more stable to transport vibration and temperature fluctuation on storage. They may be readily produced by simply mixing the components without energetic homogenisation which could in some cases be detrimental to the physiologically active substances. These factors are of considerable economic value. In addition, the solutions can be prepared to have viscosity levels permitting easy handling and injection.

Surprisingly, considerable quantities, for example of the order of 20% w/v or more, of aqueous solutions or suspensions of physiologically active materials may be held in the compositions according to this invention and substantially non-viscous compositions, suitable for injection through a narrow bore needle, may be prepared quite simply, without tre need for expensive equipment for particle size reduction and control in the case of suspensions, or homogenising machinery in the case of emulsions, which processes are difficult to control. However, thicker compositions may be obtained if required, by varying the nature of the wetting agent and lipophilic dispersion medium.

The compositions according to the invention may thus include one or more physiologically active high molecular weight substances. These substances will preferably be non-dialysable, their molecular weight being preferably above 1000, and they include, for example, antigenic substances, e.g., bacterial toxoids, proteins, polysaccharides or nucleic acids. The microorganisms which may be present can be, for example, living or dead bacteria or viruses. Thus, for example, in the veterinary field, the compositions represent an extremely useful vehicle for multiple-component vaccines for sheep and other animals, for example Brucella abortus vaccine, infectious bronchitis virus vaccine, or Clostridial vaccines, such as Cl. welchii types B, C and D, Cl. oedematiens, Cl. chauvoei, Cl. tetani and Cl. septicum.

The amount of hydrophilic oil-insoluble substances that can be dissolved in the compositions according to this invention will depend in each case on the components that are present, especially the surface-active components.

Frequently the use of more than one surface-active agent in a mixture of the appropriate H.L.B. will enable more hydrophilic material to be dissolved than would be the case using a single surface-active agent of the same H.L.B. value and it is particularly advantageous to add further to a suitable mixture of surface-active agents a surface liquidiser. Such surface liquidisers will usually be amphiphilic substances of shorter chain length than the principal surfactant and may be thought of as exerting a 'lubricating' effect by becoming interposed between the longer amphiphilic molecules. Mixtures according to this invention containing such surface liquidisers are usually considered to contain larger aggregates in the range 100—800 Å.

Such amphiphilic surface liquidisers include physiologically acceptable fatty acids, aldehydes, ketones and, in particular, amphiphilic alcohols, for example mono-, di or polyhydric alcohols having 3—10 carbon atoms. e.g. *n*-decanol, 2-ethyl-hexane-3-diol or 4-methyl-cyclohexanol.

The weight ratio of added surface liquidiser to total amphiphilic surface-agent advantageously employed to obtain transparent stable solutions is strongly dependent on the temperature range over which clarity is required. The amount is also dependent on the nature of the oil and other amphiphilic surface-active agents employed. In our preferred mixtures we have found the percentage by weight of the total surface active agent for clarity at body temperature advantageously to be not more than 40%, preferably from not more than 25%. It may be noted that the minimum quantity of amphiphilic surface-active material required to produce a clear solution may often be lower when a surface liquidiser is present.

As indicated above, the new vehicle used in the composition according to the invention is optically clear and is capable of holding considerable quantities of hydrophilic substances in a clear solution in oil. Such solutions may readily be prepared in a thin non-viscous form suitable for injection in contrast to the conventional water-in-oil emulsions which are usually too thick to be easily injected. However, by varying the nature of the wetting agent and the lipophilic dispersion medium, thicker solutions may be obtained.

The lipophilic dispersion medium may, for example, be an oil which is liquid at body temperature. In general, however the lipophilic component is preferably liquid at 35°C, more preferably at room temperature and below, to facilitate handling of injectable preparations.

The lipophilic material may thus be an aliphatic hydrocarbon, including branched chain and cycloaliphatic hydrocarbons or mixtures thereof, for example *n*-dodecane or *n*-hexadecane. Purified paraffin oil and squalane

are particularly useful examples of this class. Other lipophilic materials include natural or synthetic long chain esters or mixtures thereof such as tridecyl myristate, n-octyl or vegatable oils such as coconut oil.

When the lipophilic material is an ester or a straight or branched chain aliphatic hydrocarbon, such as paraffin oil or squalance, the surface active material preferably possesses an HLB (hydrophile-lipophile balance) value in the range 7 to 12, advantageously between 8 and 11, the optimum value being between 8.5 and 10. It should be noted that where a mixture of surface agents is used, it is the HLB value of the mixture which should fall within the above range.

The preferred surface active agents fall in the following four classes:

1) Fatty acid esters of sugar alcohol anhydrides, for example of sorbitan or mannitan. Fatty acid moieties in such substances include oleate, stearate, laurate residues etc. Sorbitan mono-oleate and mannitan mono-oleate are especially useful and mannitan monooleate is obtainable in a "specially purified" grade widely used in injectable preparations. Commercial products of this class include Arlacel (Registered Trade Mark) A (mannitan-mono-oleate), Arlacel 80 (sorbitan monooleate) and Arlacel 20 (sorbitan mono-laurate).
2) Ethylene oxide condensates of the products of class (1). Polyoxyethylene sorbitan monooleate and mono-laurate are particularly useful. Commercial products of this class include Tween (Registered Trade Mark) 80 (polyoxyethylene (20) sorbitan mono-oleate), Tween 20 (polyoxyethylene (20) sorbitan mono-laurate), Tween 81 (polyoxyethylene (5) sorbitan mono-oleate), Tween 85 (polyoxyethylene (20) sorbitan trioleate), Tween 61 (polyoxyethylene (4), sorbitan mono-stearate) and Tween 65 (polyoxyethylene (20) sorbitan tristearate). The numerical values given in parenthesis in the nomenclature for the above products refers to the approximate number of oxyethylene The products are, in fact, always mixtures and this figure merely represents the average chain length.
3) Polyoxyethylene derivatives of alkyl phenols. The alkyl portions of such phenols preferably contain 6 to 10 carbon atoms e.g. as in octyl or nonyl groups. Products of this type having polyoxyethylene chains of varying lengths are commercially available. Commercial products in this class include Triton (Registered Trade Mark) X—15 (polyoxyethylene (1)-octyl phenol), Triton X—35 (polyoxyethylene (3)-octyl phenol) and Triton X—100 (polyoxyethylene (10-octyl phenol).
4) Polyoxyethylene, derivatives of fatty alcohols e.g. lauryl, stearyl alcohol etc. Again, materials of varying chain lengths are obtainable. Brij (Registered Trade Mark) 30, (polyoxyethylene (4)-lauryl ether) is a useful prosynthetic long chain esters or mixtures include duct in this class.

Naturally the surface active material and the lipophilic material must be campatible with the biologically active component.

The HLB values of a number of surface active materials are given in Table 1 below.

TABLE I

Surface Active Agents Examined

Name	Chemical Constitution	H.L.B.
1. Arlacel 20	Sorbitan Monolaurate	8.6
Arlacel 80	Sorbitan Monooleate	4.3
Arlacel A	Mannitan Monooleate	4.0
2. Tween 20	Polyoxyethylene (20) sorbitan monolaurate	16.7
Tween 80	Polyoxyethylene (20) sorbitan monooleate	15.0
Tween 81	Polyoxyethylene (5) sorbitan monooleate	10.0
Tween 85	Polyoxyethylene (20) sorbitan trioleate	11.0
Tween 60	Polyoxyethylene (20) sorbitan monostearate	15.0
Tween 61	Polyoxyethylene (4) sorbitan monostearate	9.6
Tween 65	Polyoxyethylene (20) sorbitan tristearate	10.5
3. Triton X—15	Polyoxyethylene (1) octyl phenol	3.6
Triton X—35	Polyoxyethylene (3) octyl phenol	7.9
Triton X—100	Polyoxyethylene (10) octyl phenol	13.4
4. Brij 30	Polyoxyethylene (4) lauryl ether	9.5

In order to obtain an optimal HLB value it is often convenient to use a mixture of a predominantly hydrophilic surface active agent with a predominantly lipophilic surface agent and Table II below shows results obtained with a number of such mixtures and also Tween 81. Weighed quantities of surfactant solutions sealed in ampoules with incremental quantities of water were mixed and examined for clarity to determine the maximum quantities of water solubilised. Other techniques show higher values and those shown are principally for the purpose of comparing the properties of the surface active agents

TABLE II

Quantities (percentage w/w) of Water Solubilised at Room Temperature

Composition Number	Surface active agents	Ratio	H.L.B.	Concentration of surface active agent (% w/w)						Oil phase
				5	10	15	20	25	30	
1	Arlacel 80 : Tween 80	70 : 30	7.5	—	<1	—	<1	—	<1	Liquid paraffin
2	,, ,,	60 : 40	8.6	—	2.3	—	5.5	—	7.5	,, ,,
3	,, ,,	50 : 50	9.6	—	1.2	—	4.5	—	4.46	,, ,,
4	,, ,,	40 : 60	10.7	—	1.2	—	3.4	—	5.5	,, ,,
5	Arlacel A : Tween 80	60 : 40	8.3	—	1.2	—	2.3	—	3.4	,, ,,
6	,, ,,	50 : 50	9.6	—	1.2	—	3.2	—	3.4	,, ,,
7	,, ,,	40 : 60	10.5	—	1.2	—	2.3	—	3.4	,, ,,

TABLE II (Continued)

Quantities (percentage w/w) of Water Solubilised at Room Temperature

Composition Number	Surface Active Agents	Ratio	H.L.B.	Concentration of surface active agent (% w/w)						Oil
				5	10	15	20	25	30	
8	Arlacel 20 : Tween 80	60 : 40	11.2	1.2	2.3	—	6.62	—	10.3	Liquid paraffin
9	,, ,,	50 : 50	11.8	<1	<1	—	2.3—5.5	—	2.3—8.45	,, ,,
10	,, ,,	40 : 60	12.5	<1	<1	—	<1	—	5.4—7.4	,, ,,
11	Arlacel 80 : Tween 80	60 : 40	8.6	0.56 only	0.56—1.1	2.21	3.24	—	—	n-octyl oleate
12	,, ,,	50 : 50	9.6	0.56 only	nil	0.56—1.1	3.3	—	—	,, ,,
13	,, ,,	40 : 60	10.7	nil	nil	2.21 only	0.56—3.25	—	—	,, ,,
14	Arlacel 80 : Tween 80	60 : 40	8.6	1.2 only	1.23—	—	0.6—4.6	—	—	Squalane
15	Tween 81	—*	10.0	nil	2.3	4.5	8.6	—	—	Liquid paraffin

From these Tables it can be seen that surface active materials based on sorbitan give especially good results in that they solubilise relatively large quantities of water. The experiments indicate that Tween 81 alone, Arlacel 80: Tween 80 (60:40) and Arlacel 20: Tween 80 (60:40) are especially effective in a liquid paraffin dispersion medium. Brij 30 is another useful surface active material. It will be noted that the HLB values of these surface combinations are all about 10.

As indicated previously, the preferred HLB values stated above are those which apply when the lipophilic medium is a straight or branched chain hydrocarbon and, especially, paraffin oil or squalance. Where other lipophilic media are selected, the optimal HLB values will differ although they can readily be ascertained by experiment.

The percentage of water in the compositions may vary widely and up to about 22.5% by weigh can be incorporated in oils such as liquid paraffin while still maintaining a clear solution. On the other hand, the association of a large quantity of water with the physiologically active material may not be necessary and as little as 0.5% by weight water or even less may be present. Where the high molecular weight component is difficult to obtain in concentrated aqueous solution, as is often the case with bacterial toxoids and particularly where a mixture of several toxoids is required, it is preferred that the percentage of water should be, for example 10 to 15% with Tween 81 as surfactant. Such percentages can be obtained by using relatively large quantities of surface active agent.

Where concentrated solutions of the active component are available, however, it may be preferred that the percentage of water be kept relatively low, for example in the range 0.5% to 7% by weight, more preferably between 2.5% and 6.0%, so that the amount of surface active agent present can be minimised. The weight ratio of water to surface active agent is preferable in the range 1:1 to 1:10, advantageously 1:5 to 1:7, for example about 1:5.

In the field of human medicine, physiologically active material which may be incorporated into the composition includes tetanus, diptheria and staphylococcal toxoids as well as suspensions of organisms such as B. pertussis, V. cholerae, and influenza virus

The compositions of the invention may be prepared in a number of ways. As indicated above, it is possible for the surface active agent to be dissolved in the oil and for the aqueous material to be added thereto, preferably slowly. It is also possible to mix the aqueous components with the hydrophobic phase and to add surface active material to produce solubilisation. The surface active agent may also be added first to the aqueous component and the hydrophobic phase mixed subsequently therewith. One further method is especially useful where the physiologically active material is available only in dilute solutions, namely to prepare an emulsion of the aqueous and hydrophobic components using insufficient surface active agent to solubilise all the water and then to evaporate off a proportion of the water to leave a solubilised preparation. Evaporation of the water can be effected, for example, by passing a current of sterileair over the surface of the agitated emulsion or by evaporation at low temperatures under high vacuum.

The compositions according to the invention may, if desired contain additional components such as bacteriostatics and antiseptics or thickening agents for viscosity control.

The solubilised compositions according to the invention can in some cases be modified by heating to relatively high temperatures, for example above 40°C, whereby water separates out to give a turbid appearance. It is preferred, therefore, that the compositions remain clear on heating to at least body temperature. It should be borne in mind that some animals have a relatively high body temperature, for example the body temperature of sheep is normally around 40°C.

The new compositions according to the invention are intended for pharmaceutical and veterinary use. The virtually clear compositions according to the invention in addition to the physical advantages described above have also shown surprisingly marked adjuvant effects on the properties of the active material. Thus, for example, in the case of Cl. welchii, type D formol toxoid, the height of the antibody response was increased in our experiments by a factor of ten and the duration of protection was also increased. In the administration of veterinary vaccines, and indeed human vaccines, it is important that the duration of protection be as long as possible and if the number of injections necessary to give protection can be minimised this is of great benefit in reducing the cost of protecting large numbers of animals. While we do not wish to be bound by theoretical considerations, it is believed that the increased effectiveness of the active material is due to delayed release from the lipophilic medium.

For the better understanding of the invention the following Examples are given by way of illustration only:—

EXAMPLE 1

	Percentages
Arlacel 80 (sorbitan mono-oleate)	5.0 w/v
Tween 20 (polyoxyethyelen (20) sorbitan monolaurate)	3.25 v/v
Clostridium welchii type D, purified formol toxoid of a potency of 4,500 Lf/ml	1.45 v/v
Puremor (Registered Trade Mark) (extra light white paraffin oil) to	100 by volume

Method of preparation

1. A solution of 10 grams of Arlacel 80 in sufficient Puremor to produce 191 ml was sterilised by passage through a membrane filter.
2. Tween 20 was sterilised by autoclaving at 10 p.s.i.
3. Tween 20 (3.25 ml.) was aseptically measured into 95.5 ml. of sterile Arlacel 80 solution, and the toxoid solution added. The mixture was stirred until homogeneous and packed.

EXAMPLE 2

	Percentage
Tween 81 polyoxythylene (5) sorbitan monooleate	10.0 w/v
Clostridium welchii type D, purified formol toxoid solution containing 4,000 Lf/ml.	1.25 vv
Puremor extra light white paraffin oil to	100 by volume

Method of preparation

1. 15.0 grams of Tween 81 was dissolved in sufficient Puremor to produce 148 ml of solution. This solution was sterilised by passage through a sterile millepore membrane filter.
2. To 99 ml. of the sterile solution was added 1.25 ml. of toxoid solution. The mixture was stirred until homogeneous and packed.

EXAMPLE 3

	Percentage
Triton X—100 (polyoxyethylene (10) octyl phenol)	9.0 w/v
Triton X—15 (polyoxyethylene (1) octyl phenol)	9.0 w/v
Clostridium welchii Type D, purified formol toxoid at 4,000 Lf/ml	6.0 v/v
Puremor extra light white paraffin oil to	100 by volume

Method of preparation

1. 18.0 Triton X—15 dissolved in Puremor to produce 100 ml., was sterilised by filtration.
2. Triton X—100 was sterilised by autoclaving at 10 psi.
3. 9.0 g. Triton X—100 was mixed aseptically with 50 ml. Triton X—15 solution. The toxoid was added and the product made to 100 ml with sterile Puremor.
4. The product was then stirred until homogeneous.

EXAMPLE 4

Formula, as in Example 2.

Method of preparation

1. To a crude emulsion of toxoid solution in sterile Puremor, was added the Sterile Tween 81, stirring continually with a mechanical stirrer. The product was stirred until homogeneous.

EXAMPLE 5

Formula, as in Example 2.

Method of preparation

1. Toxoid solution was added to and mixed intimately with sterile Tween 81. This mixture was then diluted with Puremor to volume. The product was stirred until homogeneous.

EXAMPLE 6

		Percentage
Tween 81		10.0 w/v
Clostridium welchii type D toxoid at 3,600 Lf/ml		1.39 v/v
Thixin —R (glyceryl tris-12-hydroxystearate)		1.0 w/v
Puremor	to	100 by volume

Method of preparation

1. A 20% w/v solution of Tween 81 in puremor was sterilised by filtration.
2. 10 grams of Thixin -R sterilised by exposure to formalin vapour, was dispersed in sterile Puremor to produce 200 grams of suspension. The suspension was warmed to 70 C to dissolve the Thixin —R. and the mixture stirred vigorously until it had cooled to ambient temperature.
3. 1.39 ml. toxoid was added to 50 ml. of Tween 81 solution and 20 grams of Thixin gel added. The mixture was stirred until homogeneous, and diluted to 100 ml. with sterile Puremor. (Note that this vaccine was *not* clear, the Thixin —R shown in this formula was incorporated as a thickening agent, and does not produce a clear solution. It was incorporated against possible inclusion of cellular antigens together with toxoids, to determine its acceptability in terms of antitoxin response to toxoids.)

EXAMPLE 7

		Per 1 ml.	
Tween 81		0.18 g.	
Tween 80 (polyoxyethylene (20) sorbitan mono-oleate		0.02 g.	
Clostridium welchii type B purified toxoid (Formol)		100 L/f	mixture of suitable solutions to contain, for each ml of vaccine these quantities of antigens in flocculation equivalents (L/f).
Clostridium welchii type C purified toxoid (Formol)		50 L/f	
Clostridium welchii type D purified toxoid (Formol)		50 L/f	
Clostridium tetani purified formol toxoid		7.5 L/f	
Clostridium septicum purified formol toxoid		7.5 L/f	
Clostridium oedematiens type B formol toxoid		5 L/f	
Clostridium chauvoei formol culture		3×10^9	organisms.
Puremor	to	1.0 ml.	

Method of preparation
1. A mixture of toxoids to the proportions shown were freeze dried, and reconstituted in water for injection to produce 5 ml. of antigen solution per 100 ml. of vaccine.
2. A solution in Puremor containing 18 grams of Tween 81 and 2 grams of Tween 80 per 75 ml. of solution was sterilised by filtration.
3. The mixed antigen solution was added to this solution and the product made to 100 ml with sterile Puremor and stirred until homogeneous.

EXAMPLE 8

		Percentage
Tween 81		15.0 w/v
Brucella abortus strain 45/20, packed cells		5.0 w/v
Thixin — R		1.0 w/v
Puremor	to	100 by volume

Method of preparation
1. The packed cells (which contain approximately 50% water) were dispersed thoroughly in sterile Tween 81.
2. This dispersion was then dispersed in sterile Puremor 25 g. of 4% Thixin — R gel, prepared as described under example 6, was added, and the product made to volume with sterile Puremor. The product was then agitated until homogeneous.

EXAMPLE 9

		Percentage
Tween 80		1.5 w/v
Tween 81		13.5 w/v
Clostridium welchii type D formol toxoid at 3,000 lf/ml.		1.66 v/v
Squalene	to	100 by volume

Method of preparation
1. Tween 80 and Tween 81 were autoclaved at 10 psi for 30 min. to sterilise.
2. The Tweens were aseptically dissolved in sterile (filtered) Squalane, and the toxoid solution added. The product was mechanically stirred until homogeneous.

EXAMPLE 10

Formula		Percentage
Infectious Bronchitis virus suspension		20% v/v
Tween 81 } sterilised by filtration:		20% w/v
Puremor } sterilised by filtration:	to	100% by volume

Method of Preparation (using sterile equipment and aseptic technique)
1. 42.5 ml of a 23.55 per cent w/v solution of Tween 81 in Puremor was measured into a jacketed glass vessel, equipped with a ground glass lid and magnetic stirrer.
2. 10 ml. of Virus suspension was added and stirred to produce a water in oil emulsion.
3. A glass freeze drying trap containing a 'Drikold (Registered Trade Mark)—IMS" mixture was inserted in the socket provided in the lid of the vessel.

4. Vacuum, approximately 28 inches of mercury, was applied to the system, and water at 27°C circulated through the jacket. Water was condensed from the system, stirring continually, until the product was clear. The product was made to 50 ml with Puremor.

The moisture content of the product was measured as 11.7 per cent w/w.

EXAMPLE 11

Formula, as Example 10.

Method (Using sterile materials and equipment and an aseptic technique)

1. 42.5 ml of a 23.55 per cent w/v solution of Tween 81 in Puremor was measured into a glass vessel of approximately 150 ml. capacity equipped with a stirrer.

2. 10 ml. of virus suspension was added and the mixture stirred to produce a water in oil emulsion.

3. A stream of air, at approximately 4 litres per minute, was passed over the mixture, stirring continuously, until a clear product resulted.

EXAMPLE 12

Tetanus Vaccine

Tween 80		2.0% w/v
Tween 81		18.0% w/v
Clostridium tetani purified formol toxoid solution at 150 L.f/ml.		5.0% v/v
Puremor	to	100.0%

1. 36 g. of Tween 81 and 4 g. of Tween 80 were dissolved in sufficient Puremor to produce 190 mls. of solution. This solution was sterilised by passage through a sterile membrane filter.

2. To 95 mls. of the sterile solution was added 5 mls. of toxoid solution. The mixture was stirred until homogeneous and packed.

EXAMPLE 13

Pertussis Vaccine

Tween 81		20.0% w/v
Cell suspension containing 200 × 10, B. Pertussis organisms/ml.		5.0 v/v
Puremor	to	100.0%

1. 40 g. of Tween 81 was dissolved in sufficient Puremor to produce 190 mls. of solution. This solution was sterilised by filtration.

2. To 95 mls. of the sterile solution was added 5 mls. of cell suspension. The vaccine was stirred until homogeneous and packed.

EXAMPLE 14

Tween 60 (polyoxyethylene (20) sorbitan monostearate			10 g
Arlacel 80 (sorbitan monoleate)			10 g
2-Ethyl-1,3-hexanediol			4.8 g
Clostridium welchii tyoe B purified toxoid (Formol)	50 L/f	mixture of suitable solutions to contain, for each ml of vaccine these quantities.	11.6 ml
Clostridium welchii type C purified toxoid (Formol)	50 L/f		
Clostridium welchii type D purified toxoid (Formol)	50 L/f		
Clostridium tetani purified formol toxoid	7.5 L/f		
Clostridium septicum purified formol toxoid	7.5 L/f		
Clostridium oedematiens type B formol toxoid	5 L/f		
Puremor			47 g

Method of Preparation

1. A solution of 10 g Tween 60, 10 g Arlacel 80 and 4.8 g 2-ethyl-1,3-hexanediol in 47 g Puremor was sterilised by filtration.

2. The above solution was warmed to 40° and the mixed antigen added with stirring. The product was stirred until clear and allowed to cool.

It was clear over a range 10—45°C. (The final volume was 93 ml).

EXAMPLE 15

Tween 60			12 g
Arlacel 80			12 g
Clostridium welchii type B purified toxoid (Formol)	100 L/f	mixture of suitable solutions to contain, for each ml of vaccine these quantities.	
Clostridium welchii type C purified toxoid (Formol)	50 L/f		
Clostridium welchii type D purified toxoid (Formol)	50 L/f		
Clostridium tetani purified formol formol toxoid	7.5 L/f		
Clostridium septicum purified formol toxoid	7.5 L/f		
Clostridium oedematiens type B formol toxoid	5 L/f		
Clostridium chauvoei formol culture	3×10^5	organisms	
2-Ethyl-1,3-hexanediol			8.5 g.
Coconut oil	to		100 mls.

A mixture of toxoids to the proportions shown were freeze dried and reconstituted in water for injection to produce 6.7 mls. of antigen solution per 100 ml. vaccine.

Method of Preparation

1. A solution of 10 g Tween 60, 10 g Arlacel 80 and 4.8 g 2-ethyl-1,3-hexanediol in 47 g Puremor was sterilised by filtration.

2. The above solution was warmed to 40° and the mixed antigen added with stirring. The product was stirred until clear and allowed to cool.

WHAT WE CLAIM IS:—

1. Injectable compositions consisting of or containing water maintained in solution (as herein defined) in a physiologically acceptable lipophilic dispersion medium, which is liquid at body temperature, by means of one or more non-ionic, amphiphilic, physiologically acceptable surface active substances, said water having associated therewith one or more physiologically-active microorganisms and/or physiologically active, high molecular weight, hydrophilic oil-insoluble materials.

2. Compositions as claimed in claim 1 in which the physiologically active material is an antigenic substance.

3. Compositions as claimed in claim 1 in which the physiologically active material is a protein, polysaccharide or nucleic acid.

4. Compositions as claimed in claim 1 in which the physiologically active material includes one or more bacterial toxoids and/or killed bacteria.

5. Compositions as claimed in claim 4 in which the bacterial toxoid is a toxoid from *clostridium welchii* type B, C or D, *clostridium oedematiens, clostridium septicum* or *clostridium tetani* or killed bacteria from *Clostridium chauvoei* or *Brucella abortus*.

6. Compositions as claimed in claim 4 in which the physiologically active material is diphtheria or staphylococcal toxoid or B.pertussis cells.

7. Compositions as claimed in any of the preceding claims in which the lipophilic dispersion medium is liquid at room temperature.

8. Compositions as claimed in claim 7 in which the lipophilic dispersion medium is a straight or branched chain aliphatic or cycloaliphatic or a mixture of such hydrocarbons.

9. Compositions as claimed in claim 8 in

which the hydrocarbon is purified paraffin oil or squalane.

10. Compositions as claimed in claim 7 in which the lipophilic dispersion medium is a natural or synthetic long chain ester or mixtures thereof.

11. Compositions as claimed in claim 10 in which the ester is tridecyl myristate or *n*-octyl oleate or a vegetable oil.

12. Compositions as claimed in claim 11 in which the vegetable oil is coconut oil.

13. Compositions as claimed in any of the preceding claims in which the amphiphilic surface active material is a fatty acid ester of a sugar alcohol anhydride, or an ethylene oxide condensate thereof, a polyoxyethylene derivative of an alkyl phenol, a polyoxyethylene derivative of a fatty alcohol or a mixture of such materials.

14. Compositions as claimed in claim 13 in which the surface active material is an oleate, stearate or laurate of sorbitan or mannitan.

15. Compositions as claimed in claim 13 in which the surface active material is a polyoxyethylene derivative of an alkyl phenol having 6 to 10 carbon atoms in the alkyl portion or of lauryl or stearyl alcohol.

16. Compositions as claimed in any of the preceding claims in which the HLB value of the amphiphilic surface active material is between 7 and 12.

17. Compositions as claimed in any of the preceding claims in which the HLB value of the amphiphilic surface active material is between 8 and 11.

18. Compositions as claimed in any of the preceding claims in which the HLB value of the amphiphilic surface active material is between 8.5 and 10.

19. Compositions as claimed in claim 16 in which the amphiphilic surface active material is (a) 40 parts by weight polyoxyethylene (20) sorbitan mono-oleate with 60 parts by weight sorbitan mono-oleate or sorbitan monolaurate, or (b) polyoxyethylene (5) sorbitan mono-oleate.

20. Compositions as claimed in any of the preceding claims in which the water content is between0.5 and 22.5% by weight.

21. Compositions as claimed in claim 20 in which the water content is between 10 and 15% by weight.

22. Compositions as claimed in claim 20 in which the water content is between 0.5 and 7.0% by weight.

23. Compositions as claimed in claim 22 in which the water content is between 2.5 and 6.0% by weight.

24. Compositions as claimed in any of the preceding claims in which the weight ratio of water to surface active agent is in the range 1:1 to 1:10.

25. Compositions as claimed in claim 22 in which the weight ratio of water to surface active agent is in the range 1:4 to 1:7.

26. Compositions as claimed in any of the preceding claims in which the surface material includes a surface liquidising agent.

27. Compositions as claimed in claim 26 in which the surface liquidising agent is an amphiphilic substance of shorter chain length than the principal surface active agent present.

28. Compositions as claimed in claim 27 in which the surface liquidising agent is an amphiphilic physiologically acceptable alcohol.

29. Compositions as claimed in claim 28 in which said alcohol has 3 to 10 carbon atoms.

30. Compositions as claimed in claim 28 in which the alcohol is *n*-decanol, 2-ethylhexane-1,3-diol or 4-methylcyclohexanol.

31. Compositions as claimed in any of claims 26 to 30 in which the surface liquidising agent constitutes up to 40% by weight of the total surface active material.

32. Compositions as claimed in any of claims 26 to 30 in which the surface liquidising agent constitutes up to 25% by weight of the total surface active material.

33. Compositions as claimed in any of the preceding claims which also contain one or more antibacterial, or thickening agents.

34. Compositions as claimed in any of the preceding claims substantially as herein described.

35. Compositions as claimed in any of the preceding claims substantially as herein described with reference to any of the Examples.

36. A process for the preparation of a composition as claimed in claim 1 wherein water, one or more physiologically active high molecular weight oil-insoluble materials and/or physiologically active micro-organisms, a physiologically acceptable lipophilic dispersion medium and one or more non-ionic, amphiphilic, physiologically acceptable surface active substances are mixed together to form a composition in which said water is solubilised in said lipophilic medium.

37. A process as claimed in claim 36 in which the surface active material is first dissolved in the lipophilic medium followed by admixture with the physiologically active material and water.

38. A process as claimed in claim 36 in which the aqueous components are mixed with the lipophilic phase followed by admixture with the surface active material.

39. A process as claimed in claim 36 in which a macroemulsion of the aqueous and lipophilic components is prepared with insufficient surface active agent to solubilise all the water followed by causing or allowing water to evaporate from the emulsion until a solubilised preparation is formed.

40. A process as claimed in claim 36 substantially as herein described.

41. A process as claimed in claim 36 substantially as herein described with reference to any of the examples.

42. Compositions as claimed in claim 1 whenever prepared by a process as claimed in claim 36.

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